Title	Comparison of temporal changes in components of formalin guaiacol under several storage conditions
Author(s)	Yamano, Y; Sawaki, K; Ishihara, K; Miyamoto, K; Ohno, K; Kawaguchi, M
Journal	Bulletin of Tokyo Dental College, 43(2): 61-67
URL	http://hdl.handle.net/10130/315
Right	

Original Article

COMPARISON OF TEMPORAL CHANGES IN COMPONENTS OF FORMALIN GUAIACOL UNDER SEVERAL STORAGE CONDITIONS

Yukio Yamano*, Kohei Sawaki**, Kazuyuki Ishihara***, Kazuhiko Miyamoto**, Katsumi Ohno** and Mitsuru Kawaguchi**

  * 6th Grade Student, Tokyo Dental College,
    1-2-2 Masago, Mihama-ku, Chiba 261-8502, Japan
 ** Department of Pharmacology, Tokyo Dental College,
    1-2-2 Masago, Mihama-ku, Chiba 261-8502, Japan
*** Department of Microbiology, Tokyo Dental College,
    1-2-2 Masago, Mihama-ku, Chiba 261-8502, Japan

Abstract

This study examined the effects of storage conditions such as time course, temperature, fluorescent light, and darkness on the components and antibacterial activity of formalin guaiacol (FG) used in endodontic treatment. We measured the quantities of formaldehyde and guaiacol in FG and antibacterial activities against *Staphylococcus aureus, Porphyromonas gingivalis*, and *Porphyromonas endodontalis*. The components and antibacterial activity of FG in the brown or transparent tightly sealed containers were not affected by temperature or fluorescent light throughout the 4 week test. However, in the loosely sealed containers, formaldehyde and guaiacol in FG sample decreased remarkably within one week, not only in a temperature- and time-dependent manner, but also under fluorescent light at 20°C. Furthermore, the antibacterial activities in the FG sample were significantly attenuated in parallel with the decrease in formaldehyde levels. Fluorescent light caused color changes and crystallization of FG samples in the transparent containers. These results suggest that it is important to replace fresh FG every 5 to 7 days for endodontic treatment and that, in the dental office, it is advisable to store fresh FG in tightly sealed containers every 2 weeks to maintain its efficacy.

INTRODUCTION

Formalin guaiacol (FG), which is used as a root canal treatment, contains formaldehyde, guaiacol, and ethanol, which have an antibacterial activity, a sedative effect, and a solvent property, respectively. Some of these components are very volatile, so that it is critical to preserve the material appropriately for effective treatment. Several studies have shown that some anaerobic bacteria, especially *Porphyromonas gingivalis* and *Porphyromonas*

endodontalis, are closely related to the presence of acute symptoms in endodontic infections[1,6,7,16]. A change of the major components of FG may have an undesirable effect on antibacterial activity against endodontic pathogens and a sedative effect. It has been reported that the quantity of formaldehyde in formalin cresol, which is a root canal disinfectant, changed under storage conditions, *e.g.*, container, period, and temperature[10,14]. Clearly, the storage of drugs, especially drugs containing volatile components, is very important in maintaining their efficiency. In this study, we investigated the effects of various storage conditions on the components and the antibacterial activity of FG, based on the results from questionnaires on the management of FG from approximately 200 dentists in Japan.

MATERIALS AND METHODS

1. Chemicals

The following chemicals were used: FG (Neo Dental Chemical Products Co., Ltd., Tokyo, Japan), guaiacol (Wako Pure Chemical Industries Ltd., Osaka, Japan), formaldehyde (Wako Pure Chemical Industries Ltd.), 2,4-dinitrophenylhydrazine (DNPH; Nacalai Tesque Inc., Kyoto, Japan), hemin (Sigma Chemical Co., St. Louis, MO, USA), menadione (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), defibrillated horse blood (Kojinbio, Tokyo, Japan), and Trypticase soy agar (Becton Dickinson Systems Co., MD, USA). All other chemicals used were of analytical reagent grade.

2. Glass containers

Two types of glass containers (5 ml) with screw caps were prepared for storing FG samples: one was brown, and the other was transparent (colorless). FG was divided into 0.5 ml aliquots and put into tightly or loosely sealed containers. A 0.5 ml aliquot of FG contains 75 mg formaldehyde, 200 mg guaiacol, 100 mg ethanol, 20 mg methanol, and 105 mg water. In the temperature test, the brown containers with/without tight seals were stored in a dark place at 4°, 20°, 30°, or 37°C. In the light test, the brown or transparent containers with/without tight seals were placed under fluorescent light at 15 W at 20°C. Throughout the 4 weeks of tests, the FG samples were sampled at one week intervals and used to measure the quantities of guaiacol and formaldehyde and their antibacterial activities.

3. Analysis of guaiacol and formaldehyde in FG

FG solutions were diluted a hundred fold with methanol for measuring the quantities of guaiacol and formaldehyde. For guaiacol analysis, 10 μl of diluted sample was directly injected into high performance liquid chromatography (HPLC)[15]. For formaldehyde analysis, 100 μl of diluted sample was mixed with 100 μl of 2 mM DNPH dissolved in 0.5 M sulfuric acid[13]. After 1 min, 10 μl of this mixture was injected into the HPLC. The HPLC system consisted of a delivery pump (LC6A; Shimadzu Co., Kyoto, Japan), a reverse-phase column (Chemcosorb 7-ODS-H, 4.6 × 250 mm i.d.; Chemco Scientific Co., Osaka, Japan) maintained at 40°C, and an UV spectrophotometric detector (SPD-6A; Shimadzu Co.). The condition for measurements of guaiacol and formaldehyde is shown in Table 1. Containers were sequenced and weighed before and after filling FG (0.5 ml). Then, the altered weight of FG at each time point was calculated by the difference between the total weight and the container's own. The net quantity of guaiacol or formaldehyde included in the altered FG was calculated using the equation shown in Fig. 1.

4. Antibacterial activity test

Preparing culture media and measuring antibacterial activity were performed as described by Ishihara *et al.*[8]. Briefly, the medium consisted of Trypticase soy agar (30 g/liter) supplemented with 5 μg/ml hemin, 0.5 μg/ml menadione, and 10% defibrillated horse blood. The Trypticase soy agar was autoclaved and cooled to 50°C, and sterile hemin, menadione, and defibrillated horse blood were

Table 1 Analytical conditions for guaiacol and formaldehyde in HPLC system

	Guaiacol	Formaldehyde
Mobile phase	methanol : water (60 : 40)	acetonitrile : water (55 : 45)
Flow rate	0.8 ml/min	1.0 ml/min
UV wavelength	270 nm	350 nm

$$\text{Net quantity of FA or GA} = \frac{\text{StC}(\mu g) \times \text{AS (FA) or (GA)}}{\text{ASt (FA) or (GA)}} \times \frac{\text{altered weight of FG}}{1,000}$$

StC: standard concentration when injected into HPLC, AS: area of sample peak,
ASt: area of standard peak, (FA): formaldehyde, (GA): guaiacol, altered weight of
FG: FG quantity at each time point.

Fig. 1 A formula for calculating net weight of formaldehyde
or guaiacol at each time point.

added. The paper disc method was used to measure the antibacterial activity of FG against *Staphylococcus aureus*, *Porphyromonas gingivalis*, and *Porphyromonas endodontalis*[18]. The sterilized filter paper (6 mm, i.d.) was put into the center of Trypticase blood agar plate (90 × 15 mm) suspending bacterium at approximately 10^7 cells per ml, and 8 μl of FG sample was dropped onto it. Two *Porphyromonas* species were incubated for 96 hr at 37°C in an anaerobic chamber gassed with 10% CO_2-10% H_2-80% N_2, whereas *S. aureus* was incubated for 48 hr in an aerobic atmosphere at 37°C. The incubation was carried out on triplicate plates. The diameter in the blocking area was accurately measured using a slide caliper. Antibacterial activity was indicated by the percentage, with the zero week condition (control) taken as 100%.

RESULTS

1. Effects of storage conditions on the components of FG

In the brown or transparent tightly sealed containers, the quantities of formaldehyde and guaiacol in FG samples were not affected by temperature, fluorescent light, or darkness even after 4 weeks. When the loosely sealed containers were stored in the dark, the quantities of formaldehyde decreased in a temperature and time dependent manner (Fig. 2). The percentages remaining at 4°, 20°, 30°, or 37°C were respectively as follows: in the 1st week, 87, 92, 81, 68%; in the 2nd week, 83, 74, 70, 14%; in the 3rd week, 73, 49, 56, 5.6%; in the 4th week, 69, 30, 26, 0%. The quantities of guaiacol also decreased, but the decreasing ratios were smaller than those of formaldehyde (Fig. 3). When the brown or transparent loosely sealed containers were placed under fluorescent light at 20°C, formaldehyde and guaiacol also decreased to 30% and 72% of the control in the brown containers and to 22% and 77% in the transparent containers after 4 weeks. There were no differences in the decreases in two compounds between the dark and fluorescent light at 20°C.

2. Effects of fluorescent light on the visual changes in FG

In the brown tightly sealed containers, the appearance of the FG solution did not change under any conditions. Fluorescent light caused color changes and crystallization in FG samples in both loosely and tightly sealed transparent containers.
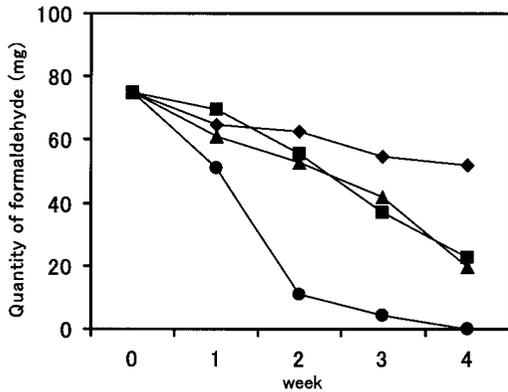
Fig. 2   Effects of temperature on the quantity of formaldehyde in FG. FG samples in the brown loosely sealed containers were kept in a dark place at 4°, 20°, 30°, and 37°C, and then the quantity of formaldehyde was measured as described in "Materials and Methods". Values are expressed as means of five samples. ◆: 4°C, ■: 20°C, ▲: 30°C, ●: 37°C.
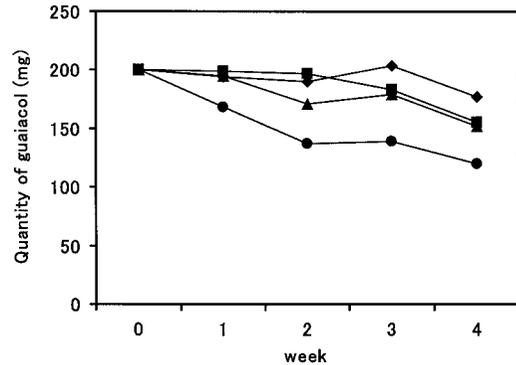

Fig. 3   Effects of temperature on the quantity of guaiacol in FG. Experimental conditions were identical to those of Fig. 2. Values are expressed as means of five samples. ◆: 4°C, ■: 20°C, ▲: 30°C, ●: 37°C.

3. Temporal changes in antibacterial activities of FG

In the brown or transparent tightly sealed containers, there were no differences in the antibacterial activities against *S. aureus*, *P. gingivalis*, and *P. endodontalis* under any conditions of temperature and fluorescent light. Figure 4 shows the results of FG samples stored under fluorescent light. However, in the loosely sealed containers, the antibacterial activities were significantly attenuated in parallel with the decrease in formaldehyde levels (Fig. 5).

DISCUSSION

In this study, we clarified that it is very important to store FG in an airtight container shaded from light for preventing loss of components and antibacterial activity.

In the temperature test, storage in the loosely sealed containers at 37°C rapidly reduced the quantity of formaldehyde and attenuated the antibacterial activity against endodontic pathogens, *P. gingivalis* and *P. endodontalis* (Figs. 2 and 5). The quantities of guaiacol also decreased in a temperature

and time dependent manner, but the decreasing ratios were smaller than those of formaldehyde (Fig. 3). This result indicates that guaiacol is relatively more stable to temperature than formaldehyde. In the tightly sealed containers, however, the quantities of formaldehyde and guaiacol did not decrease even at 37°C for 4 weeks. These results are consistent with the view reported previously for formaldehyde in formalin cresol[10,14]. Our findings suggest that sealing the container tightly was the most significant factor in preventing loss of the volatile components in the FG and that temperature (4° to 37°C) was not a direct factor; however, it could influence loss of the volatile components.

Fluorescent light includes ultraviolet (253.7 nm), which can induce photochemical reactions[11]. Our findings demonstrated that fluorescent light did not affect the quantity of volatile components in the tightly sealed containers (Fig. 4); it was involved only in changes in the nature of the components such as coloring and crystallization in the transparent containers.

In the experiment examining the antibacterial activity against *S. aureus*, *P. gingivalis*, and *P. endodontalis*, the loosely sealed containers lost their effectiveness in parallel with the
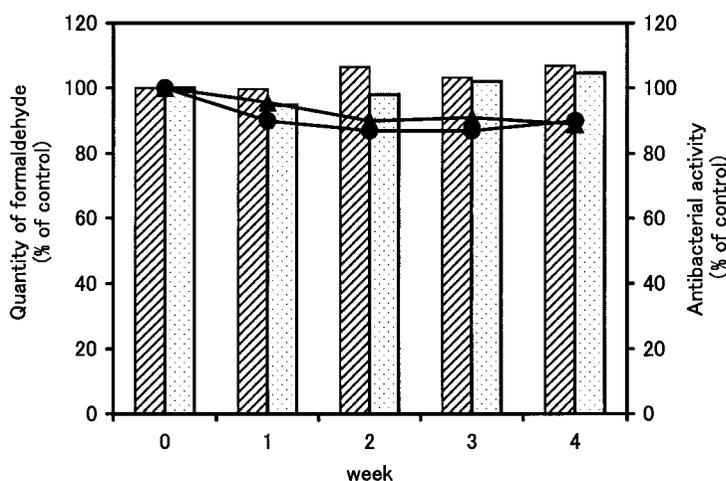
Fig. 4 Effects of fluorescent light on the quantity of formaldehyde and antibacterial activity in FG. FG samples in the brown or transparent tightly sealed containers were placed under fluorescent light at 20°C, and then the quantity of formaldehyde and antibacterial activity against *P. endodontalis* were measured as described in "Materials and Methods". Values are expressed as means of three to five samples. ▨: formaldehyde in brown container, ▥: formaldehyde in transparent container, ▲: antibacterial activity in brown container, ●: antibacterial activity in transparent container.
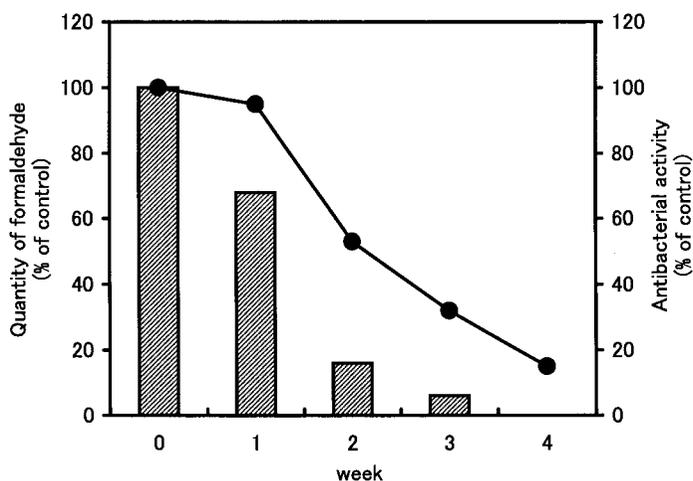

Fig. 5 Effects of temperature on antibacterial activity in FG. FG samples in the brown loosely sealed containers were kept in a dark place at 37°C, and then antibacterial activity against *P. endodontalis* was measured as described in "Materials and Methods". The quantities of formaldehyde were listed up from Fig. 2. Values are expressed as means of three to five samples. ▨: quantity of formaldehyde, ●: antibacterial activity.

decrease in the quantity of formaldehyde, which itself has a strong antibacterial activity[3,4] (Fig. 5). It is generally recognized that formaldehyde also has a tissue stimulating action[17] and a carcinogenic activity[2,9,12]. How-

ever, some research has demonstrated that the carcinogenesis of formaldehyde is closely related to dose and time dependency. Kerns *et al.*[12] reported that, when rats were exposed by inhalation to 22.4μg/kg of formaldehyde gas

6 hr/day, 5 days/week, for 24 months, only a
0.8% incidence of squamous cell carcinomas
was induced in the nasal cavities, although a
44% incidence was observed at the higher
exposure dose of $57.2 \mu g/kg$. No nasal tumors
were observed in the lower exposure groups
$(<8 \mu g/kg)$[9]. A number of studies have also
shown that formaldehyde inhalation was
weakly associated with an increase in the fre-
quency of polypoid adenomas at its lower con-
centration[2,5]. On the other hand, the dosage
and period of formaldehyde used for the den-
tal treatment are extremely smaller and
shorter than those in carcinogenic experi-
ments. Practically, when FG containing 15%
formaldehyde is used to treat a patient with
body weight of 50 kg for the endodontic treat-
ment at a dose of $5 \mu l/day$, 1 day/week, for 1
month, the total amount of formaldehyde
used is about $60 \mu g/kg$. This value corre-
sponds to a volume of $1:500$ compared to that
of *in vivo* experiments reported by Kerns *et
al.*[12] and is below the carcinogenic threshold.

Guaiacol scavenges superoxide radicals[20]
and activates the cell proliferation at very
low concentrations (10^{-12} to 10^{-10}M)[19]. This
result indicates that guaiacol greatly con-
tributes to reconstruction of the injured tis-
sues. Moreover, guaiacol may play a role of
relieving the irritation and carcinogenesis of
formaldehyde. Consequently, FG may be
regarded as a safe therapeutic agent in the
dental treatment.

From these results, we conclude that, when
being applied for endodontic treatments, we
strongly recommend that FG should be
exchanged in the root canal for fresh FG
every 5 to 7 days. Moreover, in the dental
office, it is advisable to exchange FG in a
tightly sealed container for fresh FG every 2
weeks to maintain the efficiency of FG, and it
should be kept at a low ambient temperature.

REFERENCES

1) Bogen, G. and Slots, J. (1999). Black-pig-
   mented anaerobic rods in closed periapical
   lesions. *Int Endod J* **32**, 204–210.

2) Chang, J.C., Gross, E.A., Swenberg, J.A. and
   Barrow, C.S. (1983). Nasal cavity deposition,
   histopathology, and cell proliferation after
   single or repeated formaldehyde exposures in
   B6C3F1 mice and F-344 rats. *Toxicol Appl
   Pharmacol* **68**, 161–176.

3) Cwikla, J.R. (1972). The vaporization and
   capillarity effect of endodontic medicaments.
   Oral Surg Oral Med Oral Pathol **34**, 117–121.

4) Ellerbruch, E.S. and Murphy, R.A. (1977).
   Antimicrobial activity of root canal medica-
   ment vapors. *J Endod* **3**, 189–193.

5) Feron, V.J., Bruyntjes, J.P., Woutersen, R.A.,
   Immel, H.R. and Appelman, L.M. (1988).
   Nasal tumours in rats after short-term expo-
   sure to a cytotoxic concentration of formalde-
   hyde. *Cancer Lett* **39**, 101–111.

6) Haapasalo, M. (1989). *Bacteroides* spp. in
   dental root canal infections. *Endod Dent
   Traumatol* **5**, 1–10.

7) Haapasalo, M. (1993). Black-pigmented gram-
   negative anaerobes in endodontic infections.
   FEMS Immunol Med Microbiol **6**, 213–217.

8) Ishihara, K., Takazoe, I. and Okuda, K. (1986).
   Antibiotic susceptibilities of periodontopathic
   gram-negative bacteria. *Bull Tokyo dent Coll* **27**,
   103–114.

9) Kamata, E., Nakadate, M., Uchida, O., Ogawa,
   Y., Suzuki, S., Kaneko, T., Saito, M. and
   Kurokawa, Y. (1997). Results of a 28-month
   chronic inhalation toxicity study of formalde-
   hyde in male Fisher-344 rats. *J Toxicol Sci* **22**,
   239–254.

10) Kawaguchi, M., Imoto, K., Matsumoto, Y.,
    Tsutsumi, S. and Tsuzuki, S. (1978). The time
    course on toxicity and components of
    formocresol. *Jpn J Oral Biol* **20**, 279–284. (in
    Japanese)

11) Kawaguchi, M., Ishii, Y., Yazaki, K. and
    Tsutsumi, S. (1986). Time course of l-
    epirenamine contents in a local anesthetics
    under various conditions. *Oral Therap
    Pharmacol* **5**, 23–29. (in Japanese)

12) Kerns, W.D., Pavkov, K.L., Donofrio, D.J.,
    Gralla, E.J. and Swenberg, J.A. (1983). Carci-
    nogenicity of formaldehyde in rats and mice
    after long-term inhalation exposure. *Cancer
    Res* **43**, 4382–4392.

13) Kiba, N., Yagi, R., Sun, L., Tachibana, M., Tani,
    K., Koizumi, H. and Suzuki, T. (2000).
    Poly(allylamine) beads as selective sorbent for
    preconcentration of formaldehyde and acetal-
    dehyde in high-performance liquid chromato-
    graphic analysis. *J Chromatogr A* **886**, 83–87.

14) Kuroki, K., Murakami, Y., Maeyama, H.,
    Nakamura, S., Daimon, S. and Maekawa, T.
    (1971). A study on age change of formo-
    cresol. II. Age change of formocresol mixture

in vial with respect to possible loss of form-aldehyde. *J Kyushu Dent Soc* **24**, 853–857. (in Japanese)

15) Simatupang, M.H. (1979). Simple gradient device for HPLC with variable volume and automatic regeneration. *Chromatographia* **12**, 661–664.

16) Tanner, A. and Stillman, N. (1993). Oral and dental infections with anaerobic bacteria: clinical features, predominant pathogens, and treatment. *Clin Infect Dis* **16** Suppl 4, S304–309.

17) Torneck, C.D. (1961). Reaction of hamster tissue to drugs used in sterilization of the root canal. *Oral Surg Oral Med Oral Pathol* **14**, 730–747.

18) Yasuda, H., Ninomiya, J., Kouchi, Y. and Okamoto, H. (1978). Antimicrobial studies on root canal medicament with formalin guaiacol. *Japan J Conserv Dent* **21**, 172–180. (in Japanese)

19) Yazaki, K., Mimura, T. and Kawaguchi, M. (1993). Activating effects of phenolic compounds on the osteogenic cell line. *Bull Tokyo dent Coll* **34**, 141–145.

20) Yazaki, K., Ueda, J., Ozawa, T. and Kawaguchi, M. (1999). The free radical scavenging effect of dental drugs with anisole. *Magnetic Resonance in Medicine (Japan)* **10**, 104–107. (in Japanese)

Reprint requests to:
    Dr. Mitsuru Kawaguchi
    Department of Pharmacology,
    Tokyo Dental College,
    1-2-2 Masago, Mihama-ku,
    Chiba 261-8502, Japan